Technical Data Sheet

Purified Mouse Anti- Human Topo IIα

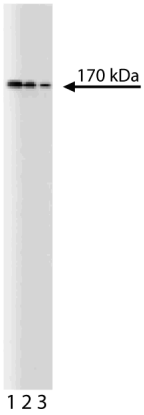
Product Information

Material Number:	**611327**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	31/Topo IIa
Immunogen:	Human Topo IIα aa. 1245-1361
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	170 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

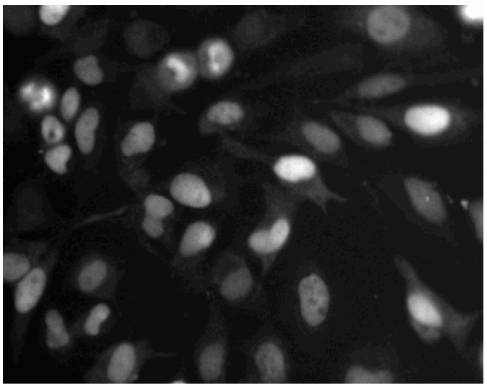
Description

Eukaryotic DNA topoisomerase II, a ubiquitous ATP-dependent type II topoisomerase, is an essential nuclear enzyme in DNA replication and transcription, chromatin segregation, and cell cycle progression. These enzymes transiently break a pair of complementary strands in double-stranded DNA to form a gate for the passage of duplex DNA. Two isoforms of DNA topoisomerase II have been identified, topo IIα and topo IIß. These have a high degree of homology, except for some divergence in the C-terminal region. Both contain multiple bipartite nuclear localization sequences (NLS) that mediate their subnuclear localization. Topo IIα levels rise during late S phase and peak in G2-M, whereas topo IIß levels remain constant throughout the cell cycle. In addition, topo IIα is expressed in proliferating cells, while topo IIß is expressed in many tissues. The exact role of these two isoforms during cell proliferation is not known, however the differences in cellular expression implicate different physiological roles. Both isoforms may also be important targets for anticancer agents that exert cytotoxicity in proliferating cells via stabilization of a topo II-DNA complex.

This antibody is routinely tested by Western blot analysis and immunofluorescent imaging. Other applications were tested at BD Biosciences Pharmingen during antibody development only.



Western blot analysis of Topo IIα on a HeLa lysate. *Lane 1: 1:500, lane 2: 1:1000, lane 3: 1:2000 dilution of the Topo IIα antibody.*



Immunofluorescent staining of HeLa cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10 000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol (see Recommended Assay Procedure) and the anti-Topo IIα antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a Pathway 850 imager using a 20x objective. This antibody also stained A549 and U2OS cells and can be used with either fix/perm protocol (see Recommended Assay Procedure).*

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20° C.

Application Notes

Application

Bioimaging	Routinely Tested
Western blot	Routinely Tested
Immunofluorescence	Tested During Development

Recommended Assay Procedure:

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
611449	HeLa Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Igs	1.0 ml	(none)
554001	FITC Goat Anti-Mouse Igs	0.5 mg	Polyclonal

Product Notices

1. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Brown MS, Holden JA, Rahn MP, Perkins SL. Immunohistochemical staining for DNA topoisomerase IIa in Hodgkin's disease. *Am J Clin Pathol.* 1998; 109(1):39-44.(Biology)

Herzog CE, Holmes KA, Tuschong LM, Ganapathi R, Zwelling LA. Absence of topoisomerase IIbeta in an amsacrine-resistant human leukemia cell line with mutant topoisomerase IIalpha. *Cancer Res.* 1998; 58(23):5298-5300.(Biology)

Mirski SE, Gerlach JH, Cole SP. Sequence determinants of nuclear localization in the alpha and beta isoforms of human topoisomerase II. *Exp Cell Res.* 1999; 251(2):329-339.(Biology)

Tsai-Pflugfelder M, Liu LF, Liu AA, et al. Cloning and sequencing of cDNA encoding human DNA topoisomerase II and localization of the gene to chromosome region 17q21-22. *Proc Natl Acad Sci U S A.* 1988; 85(19):7177-7181.(Biology)